Improvement of Conception in Sheep Using Different Hormonal Treatments during Mating and their Influence on the Antioxidant Status

Derar Refaat1* and Hamdoun2

1Department of Theriogenology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt
2Department of Animal & Poultry Production, Faculty of Agriculture, Sohag University, Sohag, Egypt

*Corresponding author: Derar Refaat, Assiut University, Faculty of Veterinary Medicine, Department of Theriogenology, Assiut, Egypt, E-mail: derar40@gmail.com and derar40@yahoo.com

Received date: May 30, 2014, Accepted date: July 14, 2014, Published date: July 19, 2014

Copyright: © 2014 Refaat D, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

The objective of this study was to compare the effects of GnRH, prostaglandin F2α (PGF2α) and oxytocin treatments at the time of natural mating on the conception rate (CR) of non lactating pluriparous ewes. All ewes (n=61) were served naturally by fertile rams every 12 hours after the beginning of estrus. After natural mating, ewes were randomly assigned into four treatment groups; G1 received PGF2α (n=14); G2 received GnRH (n=12); G3 received oxytocin (n=15) and G4 or control received placebo (n=20). Pregnancy diagnosis was performed 25 days post-insemination by transrectal ultrasonography. Ewes were bled at the day of mating and every 10 days till Day 50 post mating to determine the changes in the total antioxidants during the first third of pregnancy. Pregnancy rate was higher (P<0.05) for all the treatment groups (69.33%) compared with the control group (55.54%). Litter size did not differ between groups except for oxytocin group. Ewe lambs dominate male in this study and the sex ratio unexpectedly preferred them. Total antioxidants did not differ significantly between groups in the present study but they were at their lowest values during estrus in all the studied groups. Gestation length, birth weight, number of services, body weight did not affect the pregnancy rate. It could be concluded that treatments with GnRH and PGF and oxytocin at the time of service could improve conception rate in pluriparous ewes.

Keywords: Antioxidants; Conception; Ewe; Hormones

Introduction

Many trials have been attempted to increase fertility in ewes. Gonadotropin-releasing hormone (GnRH) and its analogues administered at the time of artificial insemination (AI) are the most common treatments in management programmers for sheep flocks [1,2]. Improvement of the conception following GnRH treatment has been attributed to the prevention of an ovulation failure or a reduced variation in the interval between the onset of estrus and ovulation [3]. However, the results are controversial after GnRH treatment of lactating cows. Many previous works reported that conception rate in cows was improved [3], while others reported no effect on pregnancy rate was obtained [1,4]. Oxytocin and PGF2α have been shown as essential parts of ovulation process [5,6] and has been known that the increase of uterine and oviduct contractility (Hawk, 1983) affects the sperm transport. There are few studies focused on the effect of PGF2α administration at the time of AI on pregnancy [6]. Oxytocin was used to increase conception rate by improving the sperm transport in the female reproductive tract of several species [7-9]. Clitoral massage which probably releases oxytocin following artificial insemination increased pregnancy in beef cows [10]. The administration of oxytocin following AI also increased CR in lactating dairy cows [9] but in another study it had hardly any effect on pregnancy in cows [11]. The objective of the present study was to study the effect of different hormonal treatments used to improve the reproductive efficiency in ewes on different reproductive parameters and antioxidant profiles after natural mating in subtopics.

Materials and Methods

This work was carried out in the Animal Production Experimental Farm, Animal and Poultry Production Department, Faculty of Agriculture, Sohag University, Egypt (latitude 28°07´N and 30°33´E)

Animals and management

Sixty one ewes Sohagi healthy, pluriparous, non parturient and non lactating ewes were used in this study. Ewes were kept away from rams before the beginning of the study and housed in semi-open pens. Ewes were fed on a concentrate mixture with wheat straw and green fodder, providing 14% crude protein and 70% total digestible nutrients during the experimental period (from September 15th till December 31st). Water was available all time. Estrus was detected using well trained teasers and personnel. Estrous ewes were mated with fertile rams every 12 hours till the end of estrus. Immediately after the last mating, animals were assigned into four groups: G1 (n=14) received 15 mg of Dinoprost IM (PGF2α, Lutalyse, Pharmacia & Upjohn, NY); G2 (n=12) received 25 µg Gonadorelin IM (Factrel, Fort Dodge, IA, USA); G3 (n=15) treated with 20 IU oxytocin IM (Biomeda-MTC Animal Health Inc., Cambridge, Ontario, Canada) and G4 (control group, n=20) received 5 ml normal saline IM. Doses and route of administration of each drug in the present study were administered according to the instructions of the manufacturers. Pregnancy was diagnosed on Day 25 post mating for all animals using a real-time, B-mode echocamera (EUB-405B, Hitachi, Tokyo, Japan) attached with a 5-7.5 MHz transducer. Visualization of a fluid-filled uterine horn with embryonic vesicles and the presence of an embryo were used as positive indicators for pregnancy. Pregnancy rate was calculated as the
number of ewes diagnosed pregnant divided by the number of mated ewes.

**Serum total antioxidants status**

Blood samples were collected from animals beginning on day 0 (day of treatment) and every 10 days till day 50 post mating. Serum was separated and stored at -20°C till assayed for total antioxidants. The total antioxidant status was measured using Total Antioxidant Capacity (TAC) Assay Kit (K274-100 BioVision, Inc. Headquarters, 155 South Milpitas Blvd., Milpitas, California 95035).

**Statistical analyses**

All statistical procedures were performed using the computational software of SAS [12]. Chi-square analysis using the PROC FREQ procedure was used to compare the pregnancy rate among the treatment groups. A t-test was used to analyze the effect of treatments on pregnancy and antioxidants concentration in the studied ewes.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gestation length</th>
<th>Ewe Body weight</th>
<th>Birth weight</th>
<th>Pregnancy rate%</th>
<th>Male births%</th>
<th>Twining %</th>
<th>Triplets %</th>
<th>litter size</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGF2α</td>
<td>154.1 ± 7.34</td>
<td>36.3 ± 3.42</td>
<td>3.5 ± 0.23</td>
<td>71±a</td>
<td>21.42</td>
<td>14.28</td>
<td>---</td>
<td>1.2</td>
</tr>
<tr>
<td>GnRH</td>
<td>157.13 ± 8.79</td>
<td>36.12 ± 5.96</td>
<td>3.71 ± 0.41</td>
<td>66±a</td>
<td>33.33</td>
<td>25.00</td>
<td>---</td>
<td>1.37</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>153.7 ± 6.32</td>
<td>38 ± 9.54</td>
<td>3.29 ± 0.52</td>
<td>71±a</td>
<td>13.33</td>
<td>26.66</td>
<td>6.66</td>
<td>1.66</td>
</tr>
<tr>
<td>Control</td>
<td>152.44 ± 0.84</td>
<td>38.29 ± 7.67</td>
<td>3.56 ± 0.43</td>
<td>55.54b</td>
<td>15.00</td>
<td>40.00</td>
<td>5.00</td>
<td>1.23</td>
</tr>
</tbody>
</table>

Table 1: Effect of different hormonal regimens on the reproductive performance of Sohagi ewes.

<table>
<thead>
<tr>
<th>Days after mating</th>
<th>PGF2α</th>
<th>GnRH</th>
<th>Oxytocin</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.31 ± 0.01a</td>
<td>0.32 ± 0.01a</td>
<td>0.25 ± 0.01a</td>
<td>0.11 ± 0.02a</td>
</tr>
<tr>
<td>10</td>
<td>4.23 ± 0.01b</td>
<td>4.04 ± 0.91b</td>
<td>5.58 ± 0.60b</td>
<td>3.22 ± 0.12b</td>
</tr>
<tr>
<td>20</td>
<td>4.29 ± 0.45b</td>
<td>5.18 ± 0.37b</td>
<td>5.27 ± 0.59b</td>
<td>4.38 ± 1.09b</td>
</tr>
<tr>
<td>30</td>
<td>5.09 ± 0.56b</td>
<td>4.66 ± 0.45b</td>
<td>4.40 ± 0.51b</td>
<td>6.23 ± 0.87b</td>
</tr>
<tr>
<td>40</td>
<td>4.99 ± 0.36b</td>
<td>5.22 ± 0.31b</td>
<td>5.36 ± 0.72b</td>
<td>4.91 ± 1.20b</td>
</tr>
<tr>
<td>50</td>
<td>7.36 ± 0.42c</td>
<td>7.06 ± 0.93c</td>
<td>6.84 ± 0.74c</td>
<td>8.34 ± 1.65c</td>
</tr>
</tbody>
</table>

Table 2: The Serum concentration of total antioxidant (mmol) in Sohagi ewes treated with different hormonal regimens.

**Discussion**

With regard to pregnancy rate, the reproductive performance of sheep in the present study improved significantly in treated ewes compared with non treated control ones and notably oxytocin treatment had a positive effect on the litter size. However, birth weight, gestation length and sex ratio as well as total antioxidants were not changed.

The present results of lambing rate in treated groups come close to the results of Beck et al. [13] who found that treatment with GnRH analogue on Day 12 post-mating increased lambing rates and litter size in ewes. In cattle, GnRH improved pregnancy rate by 7-21% [3]. This comes in inconsistency with other studies indicating that pregnancy rate was not affected by GnRH treatment following AI [1,4].

Variability in pregnancy rate among the different studies might be associated with the potency of GnRH on gonadotropin release [14] or the timing of GnRH and mating relative to the onset of estrus. Earlier studies showed that the timing of GnRH injection according to the onset of estrus affected gonadotropin release. Although exogenous GnRH at the onset of estrus increased the pre-ovulatory LH surge [3,4], conception rate increased in one study [3] but not in others [4]. However, the administration of GnRH at the time of AI, approximately 12 hours after the initiation of standing estrus, did not result in a greater surge of LH [15]. In addition, the insufficient LH surge did not have any ovulatory effect [3,15] and did not improve pregnancy. The present results indicated that administration of PGF at the time of AI following spontaneous estrus have a beneficial effect on pregnancy rate. It was suggested that a rapid increase of PGF2α in the ovary may play some important role(s) in the ovulatory process [16,17]. Others reported that prostaglandins of the E series, and
particularly PGE, play a crucial role in ovulation by determining the targeting of follicle rupture at the apex, thus allowing release of oocytes to the periovianar space [18]. A prostaglandin analogue, Cloprostenol, administration on the day of estrus of buffalo demonstrated to increase P4 levels on day 11, probably via ET-1 and Ang-II genes inhibition. It has been hypothesized that this phenomenon may be due to specific changes in genes expression, which prevent the intraluteal production of these molecules [6]. If this hypothesis is accepted, the higher pregnancy rates recorded in PG Group could be explained by the reduction of embryo mortality. Moreover, cloprostenol administration in our experiment may have helped ovary contraction and follicle rupture, improving ovulation synchrony. Furthermore, it has been proposed that PGF2a may exert a fertility effect, by causing LH release independent of progesterone withdrawal [19] and that PGF2a administration 30 h before GnRH, elevated the GnRH-induced LH release. It is still unclear if prostaglandin is able to act on LH release by a mechanism different from that induced by GnRH, or if it only enhances GnRH-induced LH release.

In sheep few studies showing the effect of oxytocin on pregnancy rate at the time of AI were published [9,11]. Bekoeva et al. [20] indicated that oxytocin, GnRH treatments affected conception rate in post partum ewes through increasing the level of thyroxin, Triiodothyronin, oestriadiol 17β and progesterone and suggested that the causes of depression of T4 and T3 levels after parturition in spring might be a lack of gonadotropins. Low concentration of T4 and T3 in certain phases of the post-partum period might be retroactively responsible for the decline in post-partum sexual activity in ewes. However, the study of Yildiz [9] indicated that pregnancy rate increased in lactating dairy cows after oxytocin administration just before AI, which agreed with the present findings. This could be due to changes in uterine contractility and possibly to the acceleration of sperm transport in the reproductive tract of ewes [7,8,21]. Oxytocin possibly exerted its influence by stimulating prostaglandin production [22,23]. In addition to involutory effects upon the uterus [24] prostaglandins may have acted as LH-stimulating [25] and estrogen-stimulating factors [26]. Although there was no significant differences among the experimental groups regarding the level of total antioxidant but it was worthy notable that the level of these elements was gradually increased throughout the early pregnancy period towards the end of the first trimester of the studied ewes. Changes in the antioxidant enzymatic defense could be a part of placentafe mutation to reactive oxygen species-induced oxidative stress at specific early developmental stages of pregnancy. Previous reports showed that the activities of antioxidant enzymes in the sheep corpus luteum (CL) are subject to major changes during early pregnancy, suggesting that the CL of early pregnancy may be rescued from luteolysis through increasing activities of key antioxidant enzymes and inhibition of apoptosis. Maintained levels of antioxidant enzymes in the CL throughout pregnancy may be linked to reactive oxygen species continuously generated in the steroidogenesis activity of luteal cells, and may be involved in the maintenance of luteal steroidogenic activity, cellular integrity and preventive to oxidative stress, improving pregnancy outcomes [27]. Even though the total antioxidant levels were not significantly different, some changes for single antioxidants such as vitamin E, as well as neuroendocrinology-related CART level [28].

Conclusion

In conclusion, the results suggest that the administration of GnRH, oxytocin and PGF at the time of natural mating increased pregnancy rate in subtropical ewes.

References


